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Role of Glycogen in Processes of Cerebellar Glial Cells under Conditions of Its Damage with Sodium Nitrite

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Ultrastructure of processes of glial cell, astrocytes of the molecular layer of cerebellar cortex in *Rana temporaria* frog, under conditions of damage to the cerebellum caused by NO-generating compound sodium nitrite was studied under an electron microscope. It is found that astrocytes have at least two types of processes: the first (fibrillar) primarily contained numerous fibrils and few glycogen granules and the second (granular) primarily containing glycogen granules. In the presence of NO-generating compound in toxic doses, fibrillar processes are damaged or completely degrade more rapidly than granular ones. The processes containing glycogen can protect both damaged synapses and individual synaptic buttons by forming a compact structure, wrapping, around them. We analyzed the possible role of glycogen of cerebellar glial cell processes in neuroglial interactions in the presence of sodium nitrite.

Key Words: *cerebellum; neuron; glial processes; sodium nitrite; glycogen*

The hypothesis proposed by A. I. Roitbak about the involvement of glial cells (GC) into closing temporal interneuronal contacts stimulated new studies in physiology and pathophysiology of neuroglial interaction [2]. Neuroglial, in particular, neuron-astrocyte interactions are now intensively studied, because they are activated during critical periods of development and under conditions of damage to the nervous system [5,6,11]. Recent studies showed that neurons and astrocytes mutually support the functions and survival of each other via cell-cell contacts, exchange of energy substrates and various molecular mediators involved in signal transmission [6,7,10,14].

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Astrocytes can be divided into two groups: protoplasmic (glycogen-rich) and fibrillar located in the gray and white matter, respectively. The structure and function of astrocytes from other brain compartments are less studied [5,6,10,11,14]. Glycogen present in astrocytes is considered by many authors as an energy substrate essential for their interaction with neurons [2,13].

The aim of the present study was an electron microscopic study of ultrastructure of processes of GC, astrocytes of the molecular layer of frog cerebellar cortex, the presence of glycogen in them, and its role under conditions of damage to the cerebellum caused by a NO-generating compound.

MATERIALS AND METHODS

The study was performed on 13 mature male frogs (*Rana temporaria*). The cerebellum was isolated in

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Ringer saline and incubated in Ringer saline containing 1 mM $NaNO_2$ for 1 h (series I) or stimulated with electric current (0.1 Hz, 10^{-4}-10^{-5}A for 1 h) in Ringer saline containing 1 mM $NaNO_2$ (series II). NO_2^- can generate NO upon reduction. In the control, the cerebellum was fixed immediately after isolation in Ringer saline.

The material was fixed in 2.5% glutaraldehyde on 0.1 M Na-cacodylate buffer containing 0.2% tannin and 0.3% sucrose (pH 7.2-7.4) for 1 h at 4°C with postfixation in 1% OsO_4 on the same buffer for 1 h. The material was dehydrated in ascending concentrations of ethanol, absolute alcohol, and acetone followed by embedding in epon and araldite. The sections were treated with uranyl acetate and lead citrate and analyzed under a JEM-100SX electron microscope at accelerating voltage of 90 kV.

RESULTS

In experimental series I, ultrastructure of glial processes (GP) was studied under normal conditions and after severe damage caused by $NaNO_2$ (1 mM). These conditions are comparable with those in stroke. It was shown that GP actively interacting with neurons differ by their structure. Some GP contained numerous intermediate filaments (Fig. 1) consisting of glial acidic protein, actin, vimentin, and some other proteins [7]. The filament bundles go in different directions rather chaotically, therefore longitudinally and transversely cut filaments are seen on sections. In other GP, glycogen granules predominated (Fig. 2), but simultaneous presence of filaments and glycogen was found in all processes.

Thus, at least two types of GP, fibrillar (fibrils) and granular (glycogen granules) were observed in our experiments under conditions of severe damage to the cerebellum caused by $NaNO_2$; these GP actively interacted with neurons forming neuron-glia contacts. The existence of two types of astrocytes in frog cerebellum was previously hypothesized on the basis of statistical data on different GP responses to stimulation of parallel fibers in the presence of NO [1]. Here we obtained new data and additional evidences of our assumption.

In series II, pronounced swelling of both synaptic elements (buttons and spines) and GP in the molecular layer accompanied by loss of cytoplasmic structures (mitochondria, cisterns of endoplasmic reticulum, *etc.*) was observed during $NaNO_2$ exposure against the background of electrical stimulation. Under these conditions, degradation of fibrillar GP was more pronounced than degradation of granular GP.

We previously found that GP can form spiral structures, wrappings, around the damaged synapse or buttons under conditions of damage with toxic doses of $NaNO_2$ [4]. Here we showed that only GP retaining glycogen and undamaged cytoplasm can interact with synaptic structures by forming the capsule, wrapping. Regular GP with glycogen is transformed in a spiral twisted around the button (Fig. 3, *a*). The site where

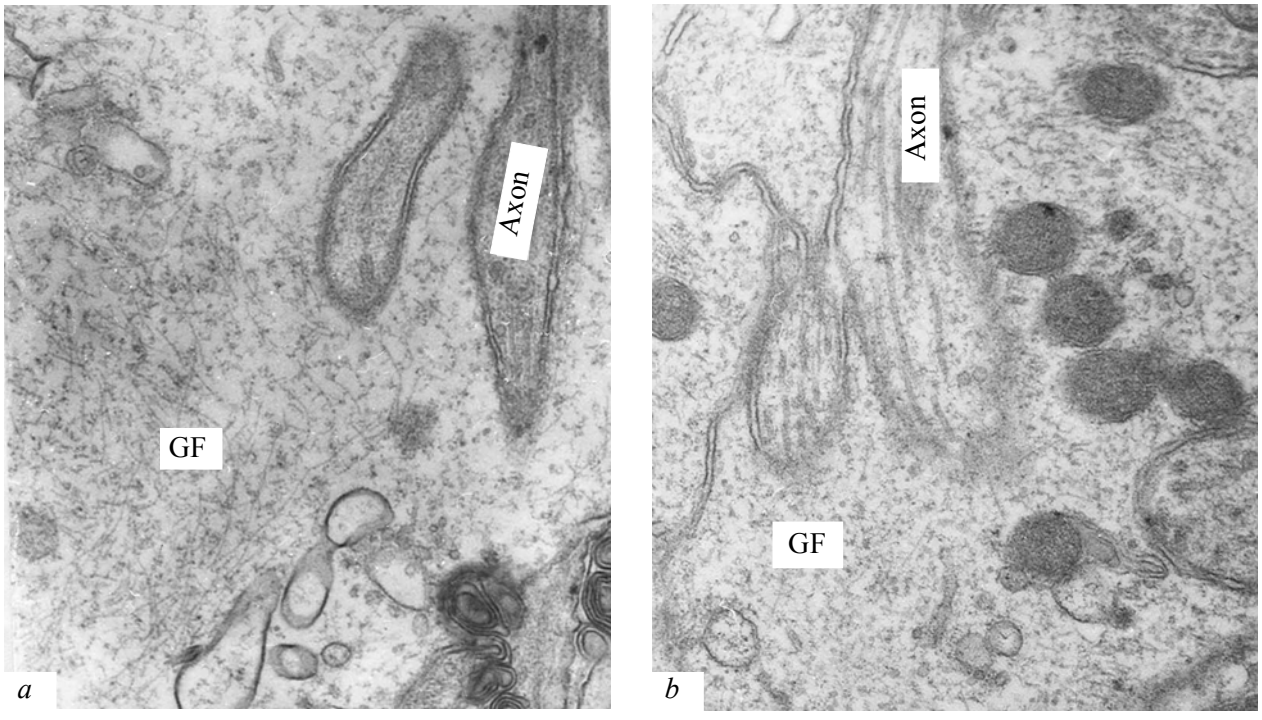

Fig. 1. Ultrastructure of a fibrillar GP after exposure to $NaNO_2$, ×35,000. *a, b*) both photographs demonstrate the presence of glial filaments (GF) in processes wrapping granular cell axons.

the process is characterized by a sharp change in its structure: the walls are narrowed and separated by transverse septae; the wrapping can consist of up to 4 layers of modified GP (Fig. 3, *b*). These wrappings most likely protect synaptic structures.

GP containing a great amount of glycogen can directly interact with synaptic vesicles [4]. Synaptic vesicles penetrate into the cytoplasm of glycogen-containing GP (Fig. 3, *c*). The integrity of glycogen-containing GP is very important under conditions of $NaNO_2$–induced damage. We can hypothesize that glycogen granules serve as energy substrate for the damaged neurons.

The functions of GP during their interaction with neurons are one of the most important problem of modern neurophysiology. GP serve as a supporting and defense apparatus for neurons [12]. They supply neurons with energy by donating glycogen and other energy substrates for Na^+/K^+-ATPases, maintain the concentration of Ca^{2+} ions and pH in the extracellular medium, regulate synapse formation and nerve pulse conduction, and protect neurons from oxidative stress [5]. GP metabolism is closely related to metabolism of

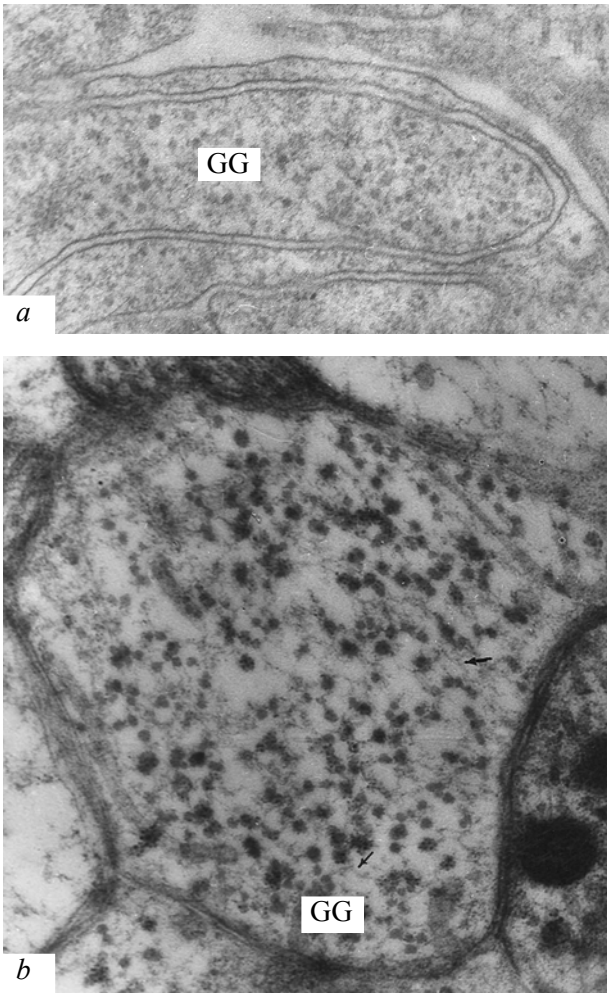

Fig. 2. Ultrastructure of a granular GP, ×70,000. *a*) GP under normal conditions, filled with glycogen granules (GG); *b*) transverse section of GP after $NaNO_2$ exposure. Numerous electron-dense glycogen granules (1 mM); arrows show glial elements.

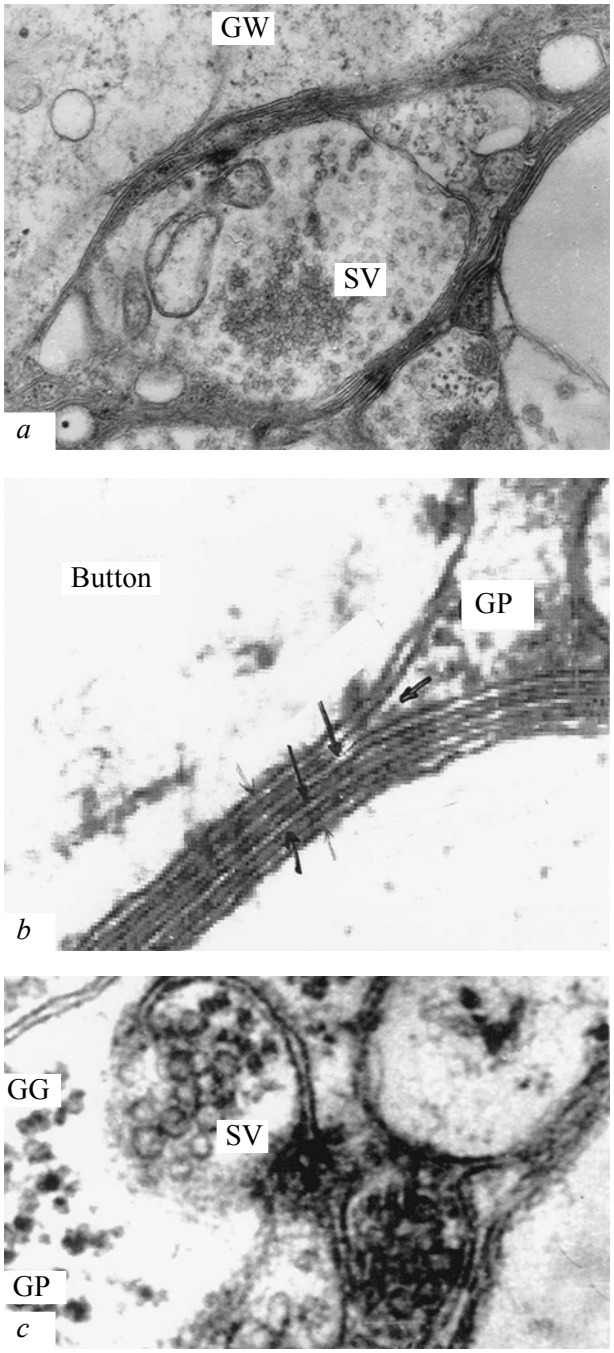

Fig. 3. Neuron-glia interaction under conditions of $NaNO_2$ exposure and electrical stimulation. *a*) glial wrapping (GW) around damaged button, synaptic vesicles (SV) are seen in it, ×30,000; *b*) a site of process transition with cytoplasm and glycogen in a layer of wrapping. Single arrows: wrapping layers; double arrows: button membrane (×110,500); c) interaction of SV with the cytoplasm of GP processes containing GG, release of SV into the cytoplasm during damage to the membrane of button and process at the same level (×90,000).

adjacent neurons. However, the functions of neuroglial interactions are poorly studied.

The appearance of glial wrappings observed by us is comparable with the results of studies on crab axon [8,9] lacking myelin sheaths. However, periaxonal sheaths in crab axons appear due to wrapping with GP containing glycogen. These glial (not containing myelin) sheaths in invertebrate are functionally analogous to myelin sheaths in vertebrates: they protect axons during electric signal conduction. Crabs are evolutionally inferior to vertebrates and some phenomena normal for them appear in vertebrates only under conditions of severe damage to neurons. Under extreme conditions, glycogen-containing astrocytic GP are activated for the realization of compensatory and adaptive reactions. Thus, astrocytic GP in vertebrates forming wrappings around synapses under conditions of their damage use the protective mechanism adopted from invertebrates.

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